Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 1, 6-8 and 18-21 are pending in the application, with claims 1 and 18 being the independent claims. Claims 7 and 18-21 have been amended taking the Examiner's comments into consideration. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Support for the amended claims may be found throughout the specification. In particular, support for claims 7 and 18-21 may be found, *inter alia*, at page 8, line 24; at page 10, line 24 and in Example 2.

Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding rejections and that they be withdrawn.

Request for Copy of All Pending Claims

In the Office Action at page 2, Examiner has requested a copy of all pending claims. Therefore, Applicants have attached to this Amendment and Reply a copy of all pending claims 1, 6-8 and 18-21.

Written Description Rejection under 35 U.S.C. § 112, First Paragraph

In the Office Action at page 2, the Examiner has maintained rejection of claims 7 and 18-21 under 35 U.S.C. § 112, first paragraph for alleged lack of written description. Applicants respectfully traverse this rejection.

The Examiner states that:

> the amendment to claims 1, 6, and 8 to recite said method using a C. glutamicum cell with a disrupted pgi gene provides sufficient written description of the claimed invention to overcome the instant rejection. However, claims 7 and 18-21 *have not* been amended as stated by applicants (page 5 of Paper No. 12, lines 3-6) to include a C. glutamicum cell with a disrupted pgi gene. Therefore, rejection of claims 7 and 18-21 is maintained for the reasons of record (see Paper Nos. 9 and 11).

Office Action at page 2. Solely to advance prosecution and not in acquiescence to the Examiner's rejection, Applicants have amended claims 7 and 18-21 to recite a disrupted *pgi* gene.

Accordingly, reconsideration and withdrawal are respectfully requested.

Enablement Rejection under 35 U.S.C. § 103(a), First Paragraph

In the Office Action at page 2, the Examiner has maintained rejection of claims 7 and 18-20 under 35 U.S.C. § 112, first paragraph for alleged non-enablement. Applicants respectfully traverse this rejection.

The Examiner states that:

> the amendment to claim 1 and 6 to recite said method using a C. glutamicum cell with a disrupted pgi gene provides sufficient enablement to overcome the instant rejection. However, claims 7 and 18-20 *have not* been amended as stated by the applicants (page 5 of Paper No. 12, lines 3-6) to narrow the scope of the clam [sic; claim] to a C. glutamicum cell with a disrupted pgi gene. Therefore, rejection of claims 7 and 18-20 is maintained for the reasons of record (see Paper Nos. 9 and 11).

Office Action at pages 2-3. Solely to advance prosecution and not in acquiescence to the Examiner's rejection, Applicants have amended claims 7 and 18-20 to recite a disrupted *pgi* gene.

Accordingly, reconsideration and withdrawal are respectfully requested.

Rejections under 35 U.S.C. § 103(a)

(A) Claims 1, 6, 7, and 18-20

In the Office Action at pages 3-5, the Examiner has maintained rejection of claims 1, 6, 7, and 18-20 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Mascarenhas *et al.*, in view of Ishino *et al.*, Voet *et al.*, and Sahm *et al.* (all documents of record). Applicants respectfully traverse this rejection.

Applicants direct Examiner's attention to Exhibit A, Wageningen University, Netherlands, Laboratory of Microbiology, figure from powerpoint presentation titled "Amino acid production: *Corynebacterium*," (printed August 27, 2002) <http://www.ftns.wau.nl/micr/collegecorynebacterium1.ppt>, which depicts six biosynthetic families of pathways in *C. glutamicum*. Exhibit A shows that tryptophan and glutamate are in pathways that are separate and distinct from the pathway that is common to lysine, threonine and isoleucine. Claims 1 and 18 (and thus the claims depending therefrom) are directed to methods of producing L-lysine, L-threonine and L-isoleucine comprising a *Corynebacterium glutamicum* cell having a disrupted *pgi* gene. Mascarenhas *et al.*, at best, discloses the deletion of a *pgi* gene in *E. coli*, and an increase in amino acids from other biosynthetic pathway families, in particular, tryptophan and glutamate. Consequently, Mascarenhas *et al.* does not disclose, suggest or otherwise contemplate the effect of a *pgi* deletion or disruption in *C. glutamicum* on the synthesis of other amino acid members outside the tryptophan and glutamate family.

Further, as neither tryptophan nor glutamate are in the same family as that of Applicants' claimed invention, such disclosure would not have led one of ordinary skill in the art to Applicants' method of producing L-lysine, L-threonine and L-isoleucine in *C. glutamicum*. Furthermore, the combination of Ishino *et al.*, Voet *et al.* and Sahm *et al.*, if

permissible, do not cure the deficiencies of Mascarenhas *et al.* There is no suggestion or motivation in the secondary art that teaches the instantly claimed invention, or that suggests that effects of inducers on any given pathway may extend to another pathway, and another amino acid biosynthetic family, as shown in Exhibit A.

Moreover, Applicants direct Examiner's attention to Exhibit B, a post-filing date document from the Howard Hughes Medical Institute-Supported Undergraduate Education in the Biological Sciences at Oklahoma State University (printed September 24, 2002), <http://opbs.okstate.edu/5853/MCA/mfa.htm>. This appears to be a report from an undergraduate research project. The report by an unknown author states:

> 2. Split at G6P - is lysine limited by NADPH, so it would benefit from more glucose going through pentose phosphate pathway? - tried pgi mutant and using gluconate instead of glucose- neither improved yield much, so limits are probably elsewhere.

See id. at ¶ A5, section 3. Thus, the Examiner errs in concluding that merely diverting carbon into the pentose phosphate pathway creates a reasonable expectation of success of induction of the biosynthesis of L-lysine, L-threonine and L-isoleucine.

In view of the foregoing remarks, Applicants respectfully assert that claims 1, 6, 7 and 18-20 are not rendered obvious by the cited art. Reconsideration and withdrawal of the rejection under 35 U.S.C. § 103(a) are therefore respectfully requested.

(B) Claims 8 and 21

In the Office Action at page 5, the Examiner has maintained rejection of claims 8 and 21 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Mascarenhas *et al.*, in

view of Ishino *et al.*, Voet *et al.*, Sahm *et al.* and in further view of Fitzpatrick *et al.* (all documents of record). Applicants respectfully traverse this rejection.

Applicants reiterate and incorporate herein the remarks made above concerning Exhibits A and B. Claims 8 and 21 are directed to methods of producing L-lysine, L-threonine and L-isoleucine comprising a *C. glutamicum* having a disrupted *pgi* gene by homologous recombination. Mascarenhas *et al.* does not disclose, suggest, or otherwise contemplate a method of producing L-lysine, L-threonine, and L-isoleucine comprising a *C. glutamicum* having a disrupted *pgi* gene via homologous recombination. Therefore, Mascarenhas *et al.* is seriously deficient as a primary reference upon which to base a *prima facie* case of obviousness because it discloses a different pathway, a different organism and a deleted *pgi* gene not by homologous recombination unlike the Applicants' claimed invention.

Furthermore, Ishino *et al.*, Voet *et al.*, Sahm *et al.* and Fitzpatrick *et al.* do not cure these deficiencies because they are silent as to increasing L-lysine, L-threonine and L-isoleucine in *C. glutamicum* by way of disrupting the *pgi* gene via homologous recombination. From Exhibit A, it is clear that separate and distinct pathways exist for at least six families of amino acids. There is no motivation or suggestion to extend the teachings of the combination of the cited art to the family of amino acids as instantly claimed.

In view of the foregoing remarks, Applicants respectfully assert that claims 8 and 21 are not rendered obvious by the cited art. Reconsideration and withdrawal of the rejection under 35 U.S.C. § 103(a) are therefore respectfully requested.

Conclusion

All of the stated grounds of rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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Version with markings to show changes made

In the Claims:

Claims 7 and 18-21 are amended as follows:

7. (Twice Amended) The method of claim 1, wherein said altered *Corynebacterium glutamicum* cell has a mutant phosphoglucose isomerase (*pgi*) gene, and wherein said mutant gene is a disrupted *pgi* gene.

18. (Twice Amended) A method of producing L-amino acids selected from the group consisting of L-lysine, L-threonine and L-isoleucine, comprising:

culturing an altered *Corynebacterium glutamicum* cell having a decreased amount of 6-phosphoglucose isomerase enzymatic activity as compared to an unaltered *Corynebacterium glutamicum* cell wherein said L-amino acid yields from said altered *Corynebacterium glutamicum* cell are greater than yields from an unaltered *Corynebacterium glutamicum* cell, and wherein said *Corynebacterium glutamicum* cell has a disrupted *pgi* gene.

19. (Twice Amended) The method of claim 18, wherein said L-amino acid yields from said altered *Corynebacterium glutamicum* cell having a disrupted *pgi* gene are from about 1% to about 100% greater than from said unaltered *Corynebacterium glutamicum* cell.

20. (Twice Amended) The method of claim 18, wherein said altered *Corynebacterium glutamicum* cell having a disrupted *pgi* gene has a mutant *pgi* gene.

21. (Twice Amended) The method of claim 18, wherein said altered *Corynebacterium glutamicum* cell having a disrupted *pgi* gene is produced by

(a) subcloning an internal region of a *pgi* gene; and

(b) inserting said resulting vector from step (a) into a *Corynebacterium glutamicum* genome via homologous recombination.